PATENT
Attorney Docket No. 010091-001

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| In re Application of | |
| Richard SCHLEGEL et al | |
| Serial No. 08/216,506 | Group Art Unit: 1813 |
| Filed: March 22, 1994 | Examiner: A. Caputa |
| For: PAPILLOMAVIRUS VACCINE | |

DECLARATION PURSUANT 37 C.F.R. § 1.132

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, JoAnn A. Suzich, Ph.D., declare and state as follows:

(1) I have been employed as a Director of the Laboratory of Biological Chemistry, at MedImmune, Inc. from 1993 to the present date.

(2) I was awarded a Ph.D. in Biochemistry from Purdue University in 1983.

(3) I am involved in virological research and papillomavirus research in particular. My curriculum vitae is attached as an exhibit to this declaration.

(4) The following experiments were conducted by myself or under my direct supervision.

**Experiment 1**

The following study provides further evidence of the importance of L1 conformational epitopes in eliciting a protective immune response to COPV. A competitive ELISA was conducted using rabbit antibodies generated against intact,

authentic COPV particles. These antibodies had been demonstrated to react with intact but not disrupted COPV virions (data not shown). In this experiment, the anti-COPV antibodies were incubated with increasing concentrations of either intact COPV L1 VLPs, COPV L1 VLPs denatured by prior incubation in bicarbonate buffer, pH 9.6 for 1 hour at 37°, or intact HPV-11 L1 VLPs. Antibody-antigen mixtures were then added to the wells of a microtiter plate which had previously been coated with purified, intact, authentic COPV virions (10 ng L1 per well). Following a 1 hour incubation period at room temperature, the plates were then washed with PBS and HRP-labeled goat anti-rabbit IgG was added. After 1 hour incubation at room temperature, the plates were washed and developed with HRP substrate. Optical density experiments were made at the 30 minute endpoint. Averages of duplicate wells were then calculated as the final optical density values. The results of this experiment can be found in Figure 1A attached to this Declaration. Neither the denatured COPV L1 VLPs nor the intact HPV-11 L1 VLPs competed for anti-COPV antibody binding. In contrast, the intact COPV L1 VLPs did compete in the assay. These results indicate that only the intact COPV L1 VLPs possessed conformational epitopes present on authentic COPV virions.

A vaccination experiment was then conducted wherein young (6-8 week old) beagles were immunized by intradermal injection with intact COPV L1 VLPs, COPV L1 VLPs which had been denatured by boiling in 1% SDS, or intact HPV-11 L1 VLPs. All immunogens were formulated in PBS. Two weeks after primary immunization,

dogs were boosted intradermally with the same dosage of an identical immunogen preparation. Two weeks after boosting, all the dogs in the study were then challenged orally with infectious COPV. Dogs were checked every week for 13 weeks after challenge for the appearance of oral warts. The results of this experiment are found in Figure 1B attached to this Declaration. These results show that the dogs which were vaccinated with the COPV L1 VLPs did not develop warts after challenge with infectious COPV. By contrast, all of the dogs which had been administered the denatured COPV L1 VLPs and HPV-11 L1 VLPs all developed warts upon challenge. Thus, only recombinant vaccine preparations which contained COPV L1 protein displaying conformational epitopes present on intact COPV virions conferred protection from COPV disease.

**Experiment 2**

A competitive ELISA was conducted as described above in Experiment 1 using intact COPV L1 VLPs, intact COPV L1 and L2 VLPs and *E. coli*-expressed COPV L1 as competing antigens. The results of this experiment are found in Figure 2A attached to this Declaration.

A second experiment was also conducted wherein dogs were vaccinated using intact COPV L1 VLPs, intact COPV L1 and L2 VLPs or *E. coli*-expressed COPV L1 as immunogens. These results are contained in Figure 2B, attached hereto. These results show that the dogs which were administered COPV L1 VLPs or COPV L1 and L2 VLPs did not develop oral warts upon challenge with infectious COPV. Both of these antigens had been demonstrated

in the competitive ELISA to possess conformational epitopes present on intact, authentic COPV virions. By contrast, the dogs which were vaccinated with non-conformationally correct COPV L1 protein expressed in *E. coli* failed to provide immunoprotection upon challenge with infectious COPV.

**Experiment 3**

The protective efficacy of VLPs comprised of L1 alone or a mixture of L1 and L2 was then compared. In this experiment groups of young (6-8 weeks old) beagles were immunized by intradermal injection with varying concentrations of immunogen contained in PBS. The various dosages administered are identified in Table 1 of this Declaration. Two weeks after primary immunization, the dogs were boosted intradermally with the same dosage of an identical immunogenic preparation. Two weeks after boosting, all the dogs in the study were then challenged orally with infectious COPV. The dogs were checked every week for ten weeks after challenge for the appearance of oral warts. The results of this comparison are contained in Table 1 of this Declaration. These results show that administration of intact COPV L1 VLPs provided for significantly better immunoprotection than intact COPV L1 and L2 VLPs. In my expert opinion, these results are surprising because endogenous COPV expresses both the L1 and L2 capsid proteins on its surface. Therefore, it would have been expected that VLPs which comprise both the L1 and L2 proteins would more effectively mimic endogenous COPV and therefore

provide for better immunoprotection upon challenge with infectious COPV.

(5) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date: 3/7/95 JoAnn A. Suzich

JoAnn A. Suzich, Ph.D.

## Biographical Sketch

JoAnn A. Suzich, Ph.D. Laboratory Director November 8, 1955

**Education:**

| | | | |
|---|---|---|---|
| Purdue University, West Lafayette, IN | Ph.D. | 1983 | Biochemistry |
| Susquehann University, Selinsgrove, PA | B.A. | 1977 | Biology |

**Research and Professional Experience:**

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| 1993 - Present | Director, Laboratory of Biological Chemistry<br>MedImmune, Inc. |
| 1990 - 1993 | Senior Scientist, Laboratory of Virology<br>MedImmune, Inc. |
| 1988 - 1990 | Staff Scientist, Laboratory of Virology<br>MedImmune, Inc. |
| 1986 - 1988 | Postdoctoral Fellow, Animal Virus Group<br>Molecular Genetics, Inc., Minnetonka MN |
| 1983 - 1986 | Postdoctoral Fellow, Department of Botany<br>University of Minnesota |
| 1977 - 1983 | Graduate Reseach Assistant, Department of Biochemistry<br>Purdue University |

**Publications:**

**Suzich, J.A.**, S. Ghim, F. Palmer-Hill, W.I. White, J.K. Tamura, J.A. Bell, J.T. Newsome, J.P. Sundberg, A.B. Jenson, and R. Schlegel. 1995. A virus-like particle based vaccine completely protects animals from disease caused by a mucosotropic papillomavirus. In preparation.

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Guilfoyle, T.J., **J.A. Suzich**, and M. Lindberg. 1986. Synthesis of 5S rRNA and putative tRNAs in nuclei isolated from wheat embryos. Plant Molecular Biology. 7:95-104.

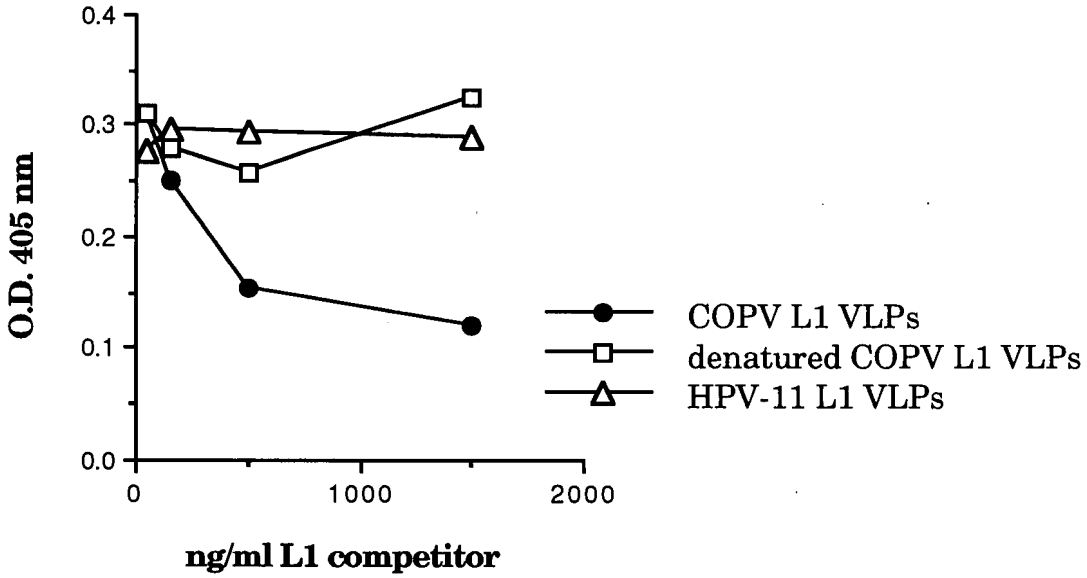
Pinto, J.E., **J.A. Suzich**, and K.M. Herrmann. 1986. 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase from potato tuber (*Solanum tuberosun* L.) Plant Physiology. 82: 1040-1044.

**Suzich, J.A.**, J.F. Dean, and K.M. Herrmann. 1985. 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase from carrot root (*Daucus carota*) is a hysteretic enzyme. Plant Physiology. 79: 765-770.

**Suzich, J.A.**, R. Ranjeva, P.M. Hasegawa, and K.M. Herrmann. 1984. Regulation of the shikimate pathway of carrot cells in suspension culture. Plant Physiology. 75: 369-371.

**A**

FIG. 1 A

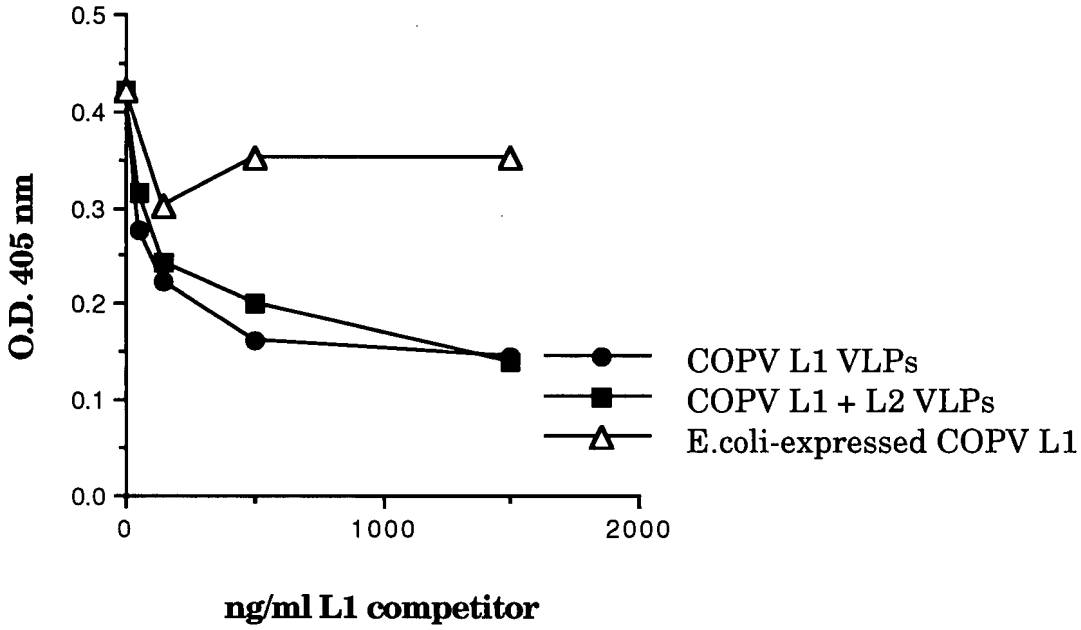



**B**

| **Immunogen** | **L1 (per dose)** | **# dogs with warts/ # dogs immunized** |
|---|---|---|
| COPV L1 VLPs | 20 ug | 0/7 |
| denatured COPV L1 VLPs | 20 ug | 7/7 |
| HPV-11 L1 VLPs | 20 ug | 7/7 |

**A**

FIG. 2A




**B**

| **Immunogen** | **L1 (per dose)** | **# dogs with warts/ # dogs immunized** |
|---|---|---|
| COPV L1 VLPs | 20 ug | 0/5 |
| COPV L1 + L2 VLPs | 20 ug | 0/5 |
| E. coli-expressed COPV L1 | 100 ug | 5/5 |

TABLE 1

| GROUP | # DOGS | IMMUNOGEN | L1 (ug per dose) | PROTECTION (#dogs with warts/#dogs in group) |
|---|---|---|---|---|
| 1 | 7 | COPV L1 VLPs | 20 | 0/7 |
| 2 | 5 | COPV L1 VLPs | 1 | 0/5 |
| 3 | 5 | COPV L1 VLPs | 0.05 | 0/5 |
| 4 | 5 | COPV L1 VLPs | 0.0025 | 2/5 |
| 5 | 5 | COPV L1 VLPs | 0.000125 | 3/5 |
| 6 | 5 | COPV L1 + L2 VLPs | 20 | 0/5 |
| 7 | 5 | COPV L1 + L2 VLPs | 1 | 0/5 |
| 8 | 5 | COPV L1 + L2 VLPs | 0.05 | 1/5 |
| 9 | 5 | COPV L1 + L2 VLPs | 0.0025 | 5/5 |
| 10 | 5 | COPV L1 + L2 VLPs | 0.000125 | 5/5 |
| 11 | 5 | PBS | --- | 5/5 |